Comparative Evaluation of Purified *Taenia solium* Glycoproteins and Crude Metacestode Extracts by Immunoblotting for the Serodiagnosis of Human *T. solium* Cysticercosis

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A lentil-lectin purified glycoprotein (LL-Gp) and a crude saline extract of *Taenia solium* metacestodes were compared for the immunodiagnosis of human cysticercosis by immunoblotting. The LL-Gp preparation was 95% sensitive for antibodies against a range of seven antigens with molecular masses of 50 to 13 kDa, whereas the sensitivity of the crude saline extract for the detection of antibodies against two major polypeptide molecules (26 and 8 kDa) was 91%. Specificity was 100% with both sets of diagnostic antigens. Affinity-purified antibodies against the 26-kDa molecule from the crude saline extract recognized the 24-kDa diagnostic region in the LL-Gp-purified extract and vice versa, suggesting that the antigens had common epitopes recognized by cysticercotic sera. In addition, in a preliminary community study of 115 randomly selected people from Bali (Indonesia), seroprevalence by immunoblot assay varied from 7.8% (with the crude saline antigen extract) to 9.6% (with the LL-Gp-purified extract). The results of this study demonstrate that both antigenic preparations are applicable for the immunodiagnosis of *T. solium* cysticercosis. The crude *T. solium* metacestode antigen extract was as specific as the purified LL-Gp *T. solium* metacestode extract and simpler to produce but slightly less sensitive.

Taenia solium taeniasis and cysticercosis occur throughout the world but principally in Latin America, the Caribbean, non-Muslim Asia, Eastern Europe, Oceania, and sub-Saharan Africa (5, 13). Cysticercosis in humans is caused by the larval (metacestode) stage of *T. solium*, which invades the subcutaneous tissues and the central nervous system (2, 3). Immunological tests for antibody detection in sera and cerebrospinal fluid are important in the diagnosis of the disease (5, 11). Presently, the use of purified or crude antigens from *T. solium* metacestodes in enzyme-linked immunosorbent assays allows a sensitive but not a specific diagnosis of human cysticercosis (8, 9). Problems include poor reproducibility, cross-reactivity with other cestodes, and questionable sensitivity because of an inappropriate contrast with normal sera (11). Currently, it appears that the best available immunoassay for the serodiagnosis of *T. solium* cysticercosis is based on the specific recognition of seven major glycoprotein bands (termed lentil-lectin purified glycoprotein [LL-Gp]) in an immunoblot assay (16, 20). This type of test has been used with good results for a number of years at the Centers for Disease Control and Prevention (CDC) (Atlanta, Ga.) and elsewhere to support the clinical diagnosis of human cysticercosis and in seroepidemiological studies (4, 14, 16). The LL-Gp test has complete specificity (100%) and high sensitivity (94 to 100%) (4, 20, 21), but a major disadvantage is the complicated nature of the antigen preparation and the cost and instability of the reagents involved in its production. Furthermore, the necessary equipment (which includes an ultracentrifuge) is often unavailable

in many laboratories in developing countries where cysticercosis is endemic. An alternative approach to preparing antigen for immunoblotting involves the use of a nonpurified saline extract of *T. solium* metacestodes (8). Specific diagnosis relies on the detection by sera from patients of two molecules of 26 and 8 kDa. This immunoblot assay was reported to have high sensitivity ($\geq 90\%$) and 100% specificity (6, 9). In the present study, nonpurified saline extract of *T. solium* metacestodes was compared with purified LL-Gp for the serodiagnosis of *T. solium* cysticercosis by an immunoblot test.

MATERIALS AND METHODS

LL-Gp and crude saline extract antigen preparation. *T. solium* metacestodes were obtained by dissecting the skeletal muscle of naturally infected pigs from Mexico.

(i) Crude *T. solium* extract. Crude antigen extract was prepared as described previously (8). Briefly, approximately 30 g of metacestodes (including vesicular fluid, cyst membranes, and scoleces) was homogenized by hand with a glass tissue homogenizer and 15 ml of phosphate-buffered saline (PBS [pH 7.2]). Homogenate was centrifuged at $14,000 \times g$ for 60 min at 4°C. Supernatant was aliquoted and stored at -20°C .

(ii) LL-Gp *T. solium*. Purified LL-Gp was prepared as described previously by Tsang et al. (20). Briefly, 20 g of frozen cysts (liquid N_2 temperatures) was quickly homogenized in 5 volumes of 5 mM HEPES-NaOH-0.25 M sucrose-0.002 M EDTA-0.05 M phenylmethylsulfonyl fluoride buffer (pH 7.2) and centrifuged at $500 \times g \times 20$ min. This supernatant was subsequently centrifuged at $250,000 \times g$ for 2 h at 4°C. Precipitate was resuspended in 30% urea and centrifuged at $48,000 \times g$. This supernatant was combined with that formed with the $250,000 \times g$ spin. Both supernatants were concentrated by ultrafiltration through a 10-kDa cutoff membrane (Amicon, Beverly, Mass.). The solution was purified by affinity chromatography through a lentil-lectin Sepharose 4B column (Pharmacia Chemicals) equilibrated with Tris-NaCl (pH 7.2). Adsorbed material was eluted with 0.2 M α -methyl-mannoside. The protein peak was concentrated and dialyzed against Tris-NaCl. This material was then aliquoted and stored at -70°C . In all cases, protein concentration was measured by a microprotein assay (Bio-Rad, Richmond, Calif.), and bovine plasma gamma globulin was used as the standard (1).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Both *T. solium* antigen extracts were separated by electrophoresis according

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to standard procedures (10) and methods described in the original publications (8, 20). Briefly, both antigen preparations were separated under nonreducing conditions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5 to 20% polyacrylamide) gradient gels on 10 by 8 cm (0.75-mm thick) minigels (Mighty Small System; Hoefer, San Francisco, Calif.). In all cases, prestained molecular-weight markers (range, 14,000 to 200,000) were employed for reference (Amersham, Buckinghamshire, United Kingdom). The optimal concentration for each antigen was defined as that which gave absolute specificity and maximum sensitivity (19). This concentration was determined by titration of the two sets of antigen preparations. All subsequent analyses were carried out at these optimal antigen concentrations, which were 100 ng/ μ l/mm for the crude antigen extract and 80 ng/ μ l/mm for the purified LL-Gp. Separated proteins were transferred electrophoretically to nitrocellulose paper (NCP) (Sartorius, Gottingen, Germany) with a miniblitter (Hoefer) at 250 mA for 2 h (18). Analyses were then carried out with human sera as originally described (8, 20). Sheets of NCP were cut into 3-mm-wide strips and incubated for 1 h with human sera diluted 1:100 in PBS (pH 7.2) containing 0.3% Tween 20 (PBST) and 5% nonfat milk. The NCP was washed with PBST three times, incubated for 1 h with horseradish peroxidase-conjugated goat antibody to human immunoglobulin G diluted 1:2,000 (Sigma Chemical Co.), and washed again with PBST three times. Antibody reactivity was visualized with a substrate solution containing 3,3'-diaminobenzidine (1 mg/ml in PBS containing 1 μ l of 30% hydrogen peroxidase per ml) (Sigma).

In a separate study, the glycoprotein antigen prepared during this study compared favorably with similar material produced at the CDC (7). The CDC acts as a reference center for cysticercosis serology in the United States, where the glycoprotein antigen was first developed.

Specific diagnosis was based on the recognition of 26- and/or 8-kDa antigens in the crude *T. solium* saline extract or at least one of the seven major glycoproteins (termed Gp50, Gp42-39, Gp24, Gp21, Gp18, Gp14, and Gp13; the prefix Gp stands for glycoprotein, and the number indicates molecular mass in kilodaltons) in the LL-Gp extract. Both assays were repeated in triplicate, with each serum and set of NCP prepared fresh each time.

Human sera. A total of 160 human serum samples, including 57 from patients with confirmed cases of *T. solium* cysticercosis, were used in this study. Thirty-one serum samples were provided by the CDC, 11 were from patients with neurocysticercosis from Yucatan (Mexico), 10 were from patients from Bali, Indonesia, whose biopsies were positive for *T. solium*, and 5 were from patients from Guatemala who had confirmed cases of neurocysticercosis. Additionally, 61 serum samples from patients infected with a variety of organisms were included as follows: *Plasmodium falciparum* (6 samples), *Trypanosoma cruzi* (2 samples), *Leishmania mexicana* (3 samples), *Trichinella spiralis* (7 samples), *Strongyloides stercoralis* (9 samples), *Onchocerca volvulus* (1 sample), *Schistosoma mansoni* (5 samples), *Echinococcus multilocularis* (which causes alveolar hydatid disease; 13 samples), *Echinococcus granulosus* (responsible for cystic hydatid disease; 9 samples), and *Taenia saginata* (4 samples) and from patients with leprosy (1 sample) and syphilis (1 sample). A negative control group included 37 individuals without evidence of cysticercosis from cysticercosis-endemic areas of Yucatan (Mexico) (17 samples) and Bali (Indonesia) (15 samples) and 5 healthy people from the United Kingdom. Additionally, four serum samples were from people with a history of epilepsy and other neurological disorders but no evidence of neurocysticercosis, and one serum sample was from a patient with a cerebral tumor and associated neurological disorders.

Serological survey of an open population. One hundred fifteen serum samples were obtained randomly from residents of Ketewel Village (Banjar Pamesan) in Bali (Indonesia), where human cases of cysticercosis had been previously diagnosed by staff at the Department of Parasitology at Udayana University. Banjar Pamesan, which had a population of 765 at the time of sampling, is 7 km east of Denpasar, the island's capital (115°12'E, 8°35'S). Individuals 1 year of age and older were selected for examination, and households were adequately informed and asked for their consent before their inclusion in the study and the collection of 5 ml of venous blood.

Sera were kept on ice until they were taken to the University of Udayana (Denpasar, Bali) and refrigerated at -20°C. Sera were brought to the United Kingdom and kept at -70°C until tested.

Immunoaffinity purification of antibodies. To determine any antigenic similarities between the two sets of antigens, immunoaffinity-purified antibodies against the 26- and 8-kDa polypeptide regions in the separated crude *T. solium* extract and Gp42-39 and Gp24 in the LL-Gp were prepared by modification of the methods of Olmsted (12) and Smith and Fisher (17). Both sets of antigens were transferred to NCP (as above). The specific regions were identified by developing three vertical strips from each end of the antigen run. Horizontal strips from each immunoblot were removed at the specific molecular-weight region and incubated overnight at 4°C with pooled cysticercotic sera ($n = 6$) or noncysticercotic control sera ($n = 6$). Antibodies bound to the horizontal strips were removed by elution with two washes of 300 μ l of 50 mM glycine elution buffer (pH 3.0) for 3 min each. The final solution was neutralized with 2 ml of PBS (pH 7.8). Aliquots from each molecular-weight region were combined, and 50 μ l of Tween 20 and 5 μ l of sodium azide were added. Eluates were equilibrated with 5 ml of PBS by ultrafiltration at 1,000 $\times g$ through a 10,000 molecular-weight cutoff (Amicon) five times for 30 min each time. A final concentrate of 400 μ l of purified antibody was stored at 4°C.

TABLE 1. Results of a comparison between crude saline and purified LL-Gp *T. solium* metacystode extracts in 160 serum samples for the immunodiagnosis of cysticercosis

Patient group and serum source or description	Confirmed test result ^a by:		No. of positive samples/total no. tested	
	CT scanning ^b	Histology	Crude saline extract	Purified LL-Gp extract
<i>T. solium</i> infected				
CDC ^c	+	NT ^d	29/31	31/31
Bali, Indonesia	NT	+	9/10	9/10
Mexico	+	NT	9/11	9/11
Guatemala	+	NT	5/5	5/5
Total			52/57	54/57
Other				
Heterologous serum samples ^e	NT	NT	0/61	0/61
Negative control	NT	NT	0/37	0/37
Patients with:				
Epilepsy and/or other neurological disorders	NT	NT	0/4	0/4
Cerebral tumor	+	NT	0/1	0/1
Total			0/103	0/103

^a +, positive diagnosis for *T. solium* cysticercosis.

^b CT, computer tomography radiological test.

^c Confirmed cysticercotic sera by similar LL-Gp immunoblot assay at CDC.

^d NT, sera not tested.

^e Heterologous serum samples, samples from patients with a variety of infectious diseases other than *T. solium* cysticercosis.

Separated pools of affinity-purified antibodies to the 26- and 8-kDa regions from the crude saline extract were used to probe 3-mm-wide strips of NCP to which either *T. solium* LL-Gp or crude extracts had been immunoblotted. Anti-Gp42-39 and Gp24 affinity-purified antibodies were used to probe similar strips. Antibody-antigen reactions were visualized as described above.

RESULTS

Comparative sensitivity and diagnostic criteria. A comparison of the crude saline and purified LL-Gp *T. solium* metacystode extracts tested with different panels of human sera is shown in Table 1.

Crude *T. solium* extract. Sensitivity was 91% (52 of 57 cysticercotic serum samples), and specificity was 100% (0 of 103 noncysticercotic serum samples). An example of individual band recognition is shown in Fig. 1A. Of the cysticercotic sera samples, 49.1% (28 of 57) recognized both the 26- and the 8-kDa regions, 42.1% (24 of 57) recognized only the 26-kDa region, and none recognized the 8-kDa region alone.

LL-Gp. With the seven glycoprotein bands described by Tsang et al. (20), 95% sensitivity (54 positive serum samples of 57 cysticercotic sera samples) and 100% specificity (0 of 103) were obtained (Table 1). No single cysticercotic serum sample reacted with all seven bands. An example of the LL-Gp results is shown in Fig. 1B; 43.9% (25 of 57 serum samples) reacted with six bands, 33.3% (19 of 57 samples) reacted with five bands, 14.0% (14 of 57) reacted with four bands, and 3.5% (2 of 57) reacted with two bands.

The sensitivity of each glycoprotein was as follows: for Gp50, 84.0% (48 of 57); for Gp42-39, 91.2% (52 of 57); for Gp24, 91.2% (52 of 57); for Gp21, 66.6% (38 of 57); for Gp18, 14.0%

FIG. 1. Western blot assays. Crude saline extract (A) and purified LL-Gp (B) from *T. solium* metacestodes. Lanes: 1 to 5, sera from patients with confirmed cysticercosis provided by CDC; 6, sera from negative control from the United Kingdom; 7, sera from negative control from cysticercosis-endemic area of Indonesia; 8, sera from patient with *T. saginata* infection; 9, sera from patient with *E. multilocularis* disease; 10, sera from patient with *E. granulosus* disease; 11 to 14, cysticercotic sera from patients from Mexico; 15, sera from negative control from cysticercosis-endemic areas of Mexico; 16, sera from patient with *T. spiralis* from Mexico; 17 and 18, sera from treated cysticercotic patients from Mexico; 19 and 20, sera from patients from Guatemala; 21, sera from patient with epilepsy and other neurological disorders from Indonesia; 22, sera from patient with cerebral tumor from Indonesia.

(8 of 57); for Gp14, 50.8% (29 of 57); and for Gp13, 73.6% (42 of 57).

Reliability. Both techniques proved to be highly reproducible. There was little, if any, variation between each of the three replicates. None of the sera from individuals with parasitic infections other than cysticercosis or with neurological disorders and none of the sera from the negative controls cross-reacted with either set of diagnostic antigens (Table 1). We were careful to measure the molecular weights of the diagnostic bands accurately, particularly with the crude extract, for which several sera samples from noncysticercotic individuals reacted with molecules close to the diagnostic regions. For instance, one serum sample from an individual with *E. multilocularis* recognized a 29-kDa region in the crude extract.

All cysticercotic individuals who were positive for the crude antigen extract were also positive for the LL-Gp. Two individuals were positive for the LL-Gp but negative for the crude antigen (these individuals recognized only Gp50, Gp18, Gp14, and Gp13).

Seroprevalence in a rural community. Results of seroepidemiological screening of 115 persons (the youngest subject tested was ≥ 1 year old, and the oldest was 78 years old) show that the rate of seropositivity measured by the LL-Gp was higher than that measured by the crude extract. Nine (7.8%) individuals were positive in both assays, and two cases were negative with the crude antigen extract but positive with the LL-Gp, recognizing only the Gp13 band. One of the individuals who were positive by both immunoblot assays was found to have subcutaneous *T. solium* cystic infection.

Antigenic similarities by affinity-purified antibodies. Figure 2 shows the antigenic similarities determined between both sets of antigens. Affinity-purified antibodies to the 26-kDa region of the crude saline extract recognized specifically the Gp24 and Gp42-39 regions of the LL-Gp. Anti-8kDa affinity-purified antibodies recognized the 26-kDa region in the crude extract but did not recognize the Gp24 region in the LL-Gp extract. Conversely, when affinity-purified antibodies to the Gp42-39 and Gp24 regions from the LL-Gp were incubated with the crude antigen extract, the antibodies to Gp24 recognized epitopes from the 26-kDa region, but the antibodies to the Gp42-39 region did not recognize any antigens in the crude saline extract.

DISCUSSION

There have been several reports of comparisons of other serodiagnostic tests for cysticercosis (enzyme-linked immunosorbent assays) with one or the other of these two immunoblot assays (4, 6, 9, 16), but the present study describes the first reported simultaneous evaluation of both of these immunoblot assays. In these two immunoblot assays, alternative methods were used for the preparation of antigen for the immunodiagnosis of *T. solium* cysticercosis. The results indicated that the semipurified glycoprotein antigen (20) was slightly more sensitive than the simpler crude antigen extract described by Gottstein et al. (8) (95% [54 of 57] compared to 91% [52 of 57]).

The sensitivities and specificities of the immunoblot assays in this study were similar to those originally reported (8, 9, 20, 21). Both antigens were over 90% sensitive with sera from patients with clinically confirmed cysticercosis. Both methods were 100% specific, showing no cross-reaction with sera from individuals with a variety of parasitic and nonparasitic diseases or neurological disorders and no cross-reaction with sera from healthy individuals.

Interpreting the results and measuring the diagnostic bands

FIG. 2. Western blot assays. (A) crude saline extract and (B) purified LL-Gp. Lanes: 1, serum from a patient with cysticercosis; 2, anti-26-kDa affinity-purified antibodies; 3, anti-8-kDa affinity-purified antibodies; 4, anti-Gp24 affinity-purified antibodies; 5, anti-Gp42-39 affinity-purified antibodies; 6, serum from negative control.

(molecular weight) had to be done carefully, particularly with the crude antigen preparation. In one case, sera from an alveolar hydatid patient reacted with a band at the 29-kDa region, which could, if mistaken for the 26-kDa band, have led to a false-positive diagnosis for *T. solium* cysticercosis.

The results also indicated that two sets of molecules had some antigenic similarities. In particular, antibodies to the Gp24 molecule recognized the 26-kDa molecule in the crude extract and vice versa. These antigens were the most sensitive molecules in their respective immunoblot assays, which might explain the high level of similarity between the two assays. In all cases, individuals who were positive to the 26-kDa molecule from the crude saline extract were also positive to the Gp24 region.

Antibodies to the 8-kDa region in the crude extract recognized the 26-kDa region from the crude extract but not the Gp24 region in the LL-Gp assay. One possible explanation is that the 8-kDa molecule might be a small subunit of one of several molecules in the 26-kDa region of the crude antigen preparation, which was not present after glycoprotein purification. Alternatively, it may be that the 26- and the 8-kDa molecules share only some epitopes. Each molecule must have its own, unique epitopes, since certain individuals were positive for only one of the two. For instance, 1 person from the community study recognized only the 8-kDa molecule, and 26 individuals who were clinically cysticercotic recognized only the 26-kDa molecule.

Besides the routine diagnosis of cysticercosis in a clinical setting, the absolute specificity of both of these antigen preparations has allowed their use in studies on open populations. The performance of less-specific tests in such circumstances is poor due to the high rate of false-positive diagnoses, resulting in poor positive predictive value. Both antigens, and in particular the LL-Gp, have been used separately in studies of the epidemiology of *T. solium* in rural communities (6, 14, 15). Schantz et al. (16) demonstrated a correlation between the LL-Gp immunoblot assay result and a clinical finding of neurocysticercosis in a rural community in Mexico where no such correlation was seen with a less-specific enzyme-linked immunosorbent assay.

The results of the current study from a *T. solium*-endemic area of Indonesia indicated that the sensitivity of the LL-Gp appeared to be slightly higher than that of the crude antigen extract. In all but one case, however, it was impossible to clinically confirm infection. One individual, positive in both assays, was subsequently diagnosed with subcutaneous *T. solium* cysticercosis. All individuals positive for the crude antigen were positive by the LL-Gp assay.

In this study, the differences between the two antigenic preparations in terms of sensitivity were not, however, statistically significant by chi-square ($P > 0.05$) (95% confidence), indicating that which one of the two tests is chosen will depend on laboratory criteria for antigen selection and other work practices.

An extension of this study will be based on the screening of an adult *T. solium* cDNA library with anti-26-kDa and anti-Gp24 affinity-purified antibodies for the identification of putative clones for 26-kDa and/or Gp24 recombinant antigens. These antigens are important, since they are consistently recognized by the cysticercotic sera of individuals in clinical and epidemiological studies (8, 15, 16, 20).

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